Effect of Different Carbon and Nitrogen Sources on the Production of Scleroglucan by an Isolate of the Fungus *Sclerotium rolfsii*

Abdul Karim Suliman Al-Noimi[1] and Mohamad Bashir Ismail Kassim[2]

[1] *Department of Biology, College of Education for Women, University of Tikrit, Tikrit, Iraq*
[2] *Department of Biology, College of Education, University of Mosul, Mosul, Iraq*

Abstract:

Effect of various carbon and nitrogen sources at different concentrations on the production of scleroglucan by an isolate of *Sclerotium rolfsii* was investigated. Results showed that maximum production of scleroglucan (5.8 g/l) was obtained when glucose was used as a carbon source particularly at the concentration of 5%. Ammonium sulfate gave maximum production of scleroglucan (6.2 g/l) when used as a nitrogen source particularly at 0.1%.

Introduction:

Many fungi known to produce extracellular polysaccharide occurred either attaching to the fungal cells as a discreet capsule or sheath (1,2) or secreted to culture medium in the form of slime (3,4). Some of these components have received much attention because of their medicinal importance in having anti-tumor activity [(5)] and their wide industrial and food application (6,7). *Sclerotium roflsii*, the cause of root rot disease of onion, was known to produce oxalic acid[(11)].

The present investigation describes the effect of various carbon and nitrogen sources on the production of scleroglucan and growth of mycelium of a strain of *S. rolfsii*.

Materials and Methods:

Scleroglucan was obtained from the fungus *Sclerotium rolfsii* which grown and subcultured previously on potato dextrose agar as described by [(8)] .

Any secretion arising were subcultured by mass hyphal transfer on PDA. an isolate was maintained of PDA and used through out this work.

The medium used for polysaccharide production had the following ingredients: KH_2PO_4, 0.5g; $MgSO_4$. $7H_2O$. 0.5g; $FeSO_4$. $7H_2O$. 0.5mg; $ZnSO_4$. $7H_2O$, 0.5mg; $FeCl_3$. $7H_2O$, 0.5mg; $CuSO_4$. $5H_2O$, 0.02 mg; $MnCl_2$. $4H_2O$, 0.02mg; $NaMO_4$. $2H_2O$, 0.02 mg; $CO(NO_3)_2$. $6H_2O$, 0.02 mg, yeat extract 2g and distilled water to make one liter of the medium.

The carbon and nitrogen sources are reported in the particular experiments. The media was adjusted to pH 6.5 and dispensed in 50 ml. Portions into 250 ml Erlenmeyer flasks and autoclaved at 1 atm.., 120°C for 20 min. The flasks were inoculated with uniform agar discs which had been cut from the margins of 7 days old colonies of *S. rolfsii* cultured on PDA medium.

The inoculated culture media were incubated with shaking at 150 rpm and 28°C for 7 days.

Sampling:

At the end of the incubation period the broth culture of each flask was diluted with 30 ml distilled water and mixed vigorously for 20 minutes with slight heating by magnetic stirrer after homogenizing.

Mycelia were collected by filtration through cheese cloth and dried at 80 °C in oven. Scleroglucan were precipitated by the addition of two volumes of ethanol to the culture fluid to precipitate it around the stirrer. The precipitate collected thoroughly and dried at 60°C in oven too. In some cases it need to be collected after complete settlement for at least 24 hrs.

Sugar residue determined by phenol sulfuric acid method [(9)] after precipitation of polysaccharides.

Results and Discussion:

Effect of Carbon Sources:

Sclerotium rolfsii strain was cultured on various carbon sources i.e. glucose, sucrose, fructose, maltose, xylose, lactose, galactose ribose, mannose and arabinose. Each carbon source was added at the concentration of 3% (w/v) to the medium. After 7 days of incubation, production of scleroglucan and growth of mycelium were determined. Table-1 showed good scleroglucan production and produced well mycelium growth on all carbon sources tested. The best carbon sources for polysaccharide production was glucose however maltose is the best for growth of mycelium.

The lowest yield was obtained when lactose, galactose and ribose were used as carbon sources. The same trend has observed with fungus *Aureobasidium pullulans* in which lactose and galactose gave the least production of pullulans among other sugars used as carbon sources(10).

Table(1) Production of scleroglucan with different carbon sources.

Carbon Sources 3% (w/v)	Biomass g/l	Scleroglucan g/l	Conversion %	Yield %	Residual Sugar g/l	Final pH
Glucose	4.78	5.3 a*	20.62	17.66	4.3	2.45
Fructose	2.26	1.6 bcd	16.0	5.33	20.0	2.7
Sucrose	9.28	3.0 ab	12.76	10.0	6.5	2.57
Maltose	9.04	3.2 ab	13.14	10.7	5.5	2.2
Xylose	2.58	0.6 d	6.31	2.0	20.5	2.38
Lactose	0.72	0.00 e	0.00	0.00	20.0	2.17
Galactose	0.19	0.00 e	0.00	0.00	23.7	2.59
Ribose	0.82	0.00 e	0.00	0.00	22.0	2.44
Mannose	7.43	2.6 abcd	15.77	8.66	13.52	2.38
Arabinose	1.16	0.8 cd	10.32	2.66	22.25	2.37

* Treatments carry similar letters did not differ significantly at 0.01 according to Duncan Multiple Rang Test.

$$\text{Conversion \%} = \frac{Scleroglucan\ \ g/l}{Consume\ \ sugar\ \ g/l} \times 100$$

$$\text{Yield \%} = \frac{Scleroglucan\ \ g/l}{Conc.\ of\ \ sugar\ \ g/l} \times 100$$

Effect of Carbon Concentration:

The fungus was cultured in various glucose concentration (1.25, 2.5, 3.75, 5.0, 6.25, 7.5, 8.75, 10.0, 11.25, 12.5 %). Table-2 shows that production of scleroglucan with the increased concentration of glucose.

Optimum concentration of glucose for maximum production of scleroglucan was /5.0%/.

Table(2) Production of scleroglucan with different concentration of glucose.

Con . of Carbon 3% (w/v)	Biomass g/l	Scleroglucan g/l	Conversion %	Yield %	Residual Sugar g/l	Final pH
1.25	2.76	1.4 c	14.73	11.2	3.0	2.23
2.5	3.80	3.12 bc	14.89	12.48	4.05	2.16
3.76	4.78	4.5 bc	12.77	12.0	6.2	2.45
5.0	5.78	5.8 abc	14.68	11.6	10.5	2.2
6.25	7.33	5.85 abc	11.48	9.36	11.55	2.15
7.5	9.34	6.0 abc	10.34	8.0	17.0	2.44
8.75	9.85	6.15 abc	8.97	7.0	19.0	2.35
10.0	10.2	7.2 ab	9.0	7.2	20.0	2.28
11.25	11.0	7.35 ab	8.21	6.43	23.0	2.33
12.5	13.3	9.3 a	9.40	7.60	24.0	2.33

$$\text{Conversion \%} = \frac{Scleroglucan\ g/l}{Consume\ sugar\ g/l} \times 100$$

$$\text{Yield \%} = \frac{Scleroglucan\ g/l}{Conc.\ of\ sugar\ g/l} \times 100$$

Effect of Nitrogen Sources:

In this experiment, various nitrogen sources were added to the media (with 0.012% nitrogen these include (ammonium sulfate, 0.1%; Sodium nitrate, 0.127%; Sodium nitrate, 0.103%; Potassium nitrate, 0.15%; lysine, 0.068%; asparagin, 0.1%; a Lenin 0.04%; ammonium phosphate, 0.174%; ammonium chloride, 0.079% and urea 0.043%).

The data in Table-3 showed clearly that scleroglucan was the lowest production and mycelium growth was achieved with best ammonium sulfate. In this aspect a polysaccharide, pendulan was known to be produced on ammonium sulfate and organic nitrogen as well(12).

Table (3) Production of scleroglucan with different Nitrogen sources.

Nitrogen Sources o.02 % (w/v)n	Biomass g/l	Scleroglucan g/l	Conversion %	Yield %	Residual Sugar g/l	Final pH
$NH_4.SO_4$	7.64	6.2 a	15.54	12.4	10.12	2.38
$NaNO_3$	3.5	1.9 b	7.85	3.8	25.8	2.6
KNO_3	6.64	1.5 b	3.87	3.0	11.25	2.7
$NaNO_2$	0.1	0.00	0.00	0.00	33.75	6.0
Lysine	2.65	0.6 b	5.52	1.2	26.25	2.35
Aspargin	6.0	1.4 b	4.82	2.8	21.0	2.62
Alinine	3.84	0.8 b	3.83	1.6	21.75	2.61
NH_4HPO_4	5.27	0.9 b	2.62	1.8	15.75	2.33
NH_4Cl	5.39	1.5 b	4.37	3.0	15.75	2.55
Urea	5.46	0.8 b	2.35	1.6	16.0	2.42

Similar letter mean no significance

$$\text{Conversion \%} = \frac{Scleroglucan\ g/l}{Consume\ sugar\ g/l} \times 100$$

$$\text{Yield \%} = \frac{Scleroglucan\ g/l}{Conc.\ of\ sugar\ g/l} \times 100$$

Effect of Nitrogen Concentration:

Sclerotium rolfsii strain was cultured in various concentration of ammonium sulfate (0.07, 0.08, 0.09, 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16%).

The best concentration for scleroglucan production was 0.1% and for growth of the mycelium was 0.16%. (0.012% as nitrogen) (Table-4).

Table (4) Production of scleroglucan with concentrations of different Ammonium sulphate.

Con. Of NH_4SO_4 % (w/v)	Biomass g/l	Scleroglucan g/l	Conversion %	Yield %	Residual Sugar g/l	Final pH
0.07	5.1	1.8 bcd	5.52	3.6	17.4	2.34
0.08	5.61	2.6 b	7.85	5.2	16.92	2.33
0.09	6.37	1.3 cd	3.74	2.6	15.32	2.31
0.10	6.95	2.2 a	13.0	10.4	10.05	1.8
0.11	7.89	1.5 bcde	3.57	3.0	8.04	1.5
0.12	8.88	1.1 de	2.60	2.2	7.74	1.5
0.13	9.4	1.0 e	2.29	2.0	6.52	1.8
0.14	9.56	1.3 cde	2.95	2.6	6.0	1.7
0.15	9.96	2.5 bc	5.66	5.0	5.88	1.7
0.16	11.06	1.4 bcde	3.15	2.8	5.61	1.5

Similar letter mean no significance

$$\text{Conversion \%} = \frac{Scleroglucan\ g/l}{Consume\ sugar\ g/l} \times 100$$

$$\text{Yield \%} = \frac{Scleroglucan\ g/l}{Conc.\ of\ sugar\ g/l} \times 100$$

References:

1. E.H. Merrifield, A.M. Stephen, Carbohydr. Res, 86:69-76 (1980).
2. J. Reiss, Trans-Br. Mycol. Soc., 56: 481-482 (1971).
3. K.W.Buck, A.W.Chen, A.G.Dickerson and E.B. Chain, J.Gen. Microbiol., 51: 337-352 (1968).
4. A. Misaki, Y. Taumuraya and S. Takaya, Agric. Biol. Chem., 42: 491-493. (1978).
5. R.L.Whistler, AA. Bushway, P.P.Singh, Adv. Carbohyd, Chem. Biochem., 32: 235-275 (1979).
6. S.Yuen, Process. Biochem., 9: 7-11 (1974).
7. J.E.Zajic and A.LeDuy "Encyclopedia of polymer scince and technology". Suppl -2-. John Wiley and Sons, N.Y., 1977, 643-652.
8. F.J.Lynch and M.J.Geoghegan, Trans. Br.Mycol. Soc.,73: 311-327. (1979).
9. M.Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, and chem., 28: 350-356 (1959).
10. S.Ueda, K.Fujita, K.Komatsu and Z.Nakashima Appl. Micobios., 11:211-215. (1963).
11. Y.Wang, B. McNeil, Scler oglucan. Critic. Rev. Biotech. 16: 185-215. (1996).
12. Y. Iwamura, M. Murata, K. Kanamaru, Y. Mikami and T. Kisski, Agric, Biol. Chem., 45: 653-657. (1981)

تأثير مصادر كاربونية ونايتروجينية مختلفة في إنتاج السكليروكلوكان من قبل أحد عزلات الفطر (*Sclerotium rolfsii*)

عبد الكريم سليمان النعيمي[1] و محمد بشير اسماعيل قاسم[2]

[1] *قسم علوم الحياة، كلية التربية للبنات، جامعة تكريت، تكريت، جمهورية العراق*

[2] *قسم علوم الحياة، كلية التربية، جامعة الموصل، الموصل، جمهورية العراق*

الملخص:

تم دراسة تأثير مصادر كاربونية ونايتروجينية وبتراكيز مختلفة على نمو الفطر *S. rolfsii* وإنتاج السكليركلوكان. أظهرت النتائج أن الكلوكوز أعطى أقصى إنتاج للسكليركلوكان (5.8 غم /لتر) خصوصا"عند التركيز (5%) بينما أعطى المصدر النتروجيني كبريتات الامونيوم أقصى إنتاج للسكليركلوكان (6.2 غم/لتر) خصوصا" عند التركيز (0.1 %).